Listeria monocytogenes in Food Outlets: Prevalence and Spread During the Purchasing Process

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Abstract: The aim of this study was to determine the prevalence of *Listeria monocytogenes* in food outlets in Saudi Arabia and study its cross-contamination of surfaces and antibiotic susceptibilities. Overall, 933 samples were surveyed, comprising 96 cutting boards, 90 conveyer belts, 216 handheld baskets and 531 plastic bags. The analysis revealed that 98/933 (10.5%) and 47/933 (5.9%) samples were positive for *Listeria* spp. and *L. monocytogenes*, respectively. In addition, the prevalence of *L. monocytogenes* was the highest on cutting boards, followed by handheld shoppers' baskets and conveyer belts. The results indicated that *L. monocytogenes* cross-contamination of items not known to be primary sources of *L. monocytogenes,* such as plastic bags, is commonplace during the purchasing process. Plastic bags from leaving shoppers were found contaminated with the offensive pathogen at a rate of 2.5%, 6% and 7% in small grocery shops, supermarkets and butcheries, respectively. Handheld baskets were contaminated with *L. monocytogenes* at a rate of 3.6% and 7.5% in small grocery shops and supermarkets, respectively. Handheld baskets were contaminated with *L. monocytogenes* at a rate of 3.6% and 7.5% in small grocery shops and supermarkets, respectively. The survival rate of *L. monocytogenes* on studied fomites was also experimentally determined for all tested surfaces and found to be the highest on cutting boards. These findings confirm the need to regularly disinfect cutting boards, conveyer belts and handheld baskets. The most contaminated surfaces, cutting boards in particular, may require regular disinfection protocols. In addition, shoppers must be made aware that their plastic bags could be contaminated with *L. monocytogenes.* Antibiotic susceptibility testing, performed on 108 *L. monocytogenes* isolates, indicated that resistance was common against augmentin, erythromycin and cloxacillin and that amoxicillin, gentamicin and chloramphenicol were the most effective antibiotics.

[Alqumber M. A. ***Listeria monocytogenes* in food outlets: Prevalence and Spread During the Purchasing Process.** *J Am Sci* 2014;10(7):148-155] (ISSN: 1545-1003). http://www.jofamericanscience.org. 20

Keywords: *Listeria monocytogenes*; food retail contamination; plastic bags; handheld baskets; conveyor belts.

1. Introduction

Gastrointestinal infections are an important health problem and a leading cause of illness and death globally (Schiller, 2009). Food-borne infections causing gastrointestinal diseases are often a result of ingesting contaminated food. Food can be contaminated when prepared by someone whose hands are not washed properly, or via contact with utensils or cutting boards that are not fully clean. *Listeria monocytogenes*, a Gram-positive rod-shaped microorganism, can cause listeriosis in humans mainly through the ingestion of contaminated foods and can result in meningitis and septicemia in neonates (Graves et al., 2005). Symptoms of listeriosis in adults usually include fever, muscle aches, vomiting and diarrhea (Graves et al., 2005). The infection can spread and cause meningitis, especially in the elderly, newborns and the immunocompromised individuals. Pregnant women are particularly susceptible to listeriosis, which may result in premature delivery, stillbirth or infection of the newborn (Mead et al., 2006). As *L. monocytogenes* also infect many animals (Felix et al., 2014), contamination of food, hands and utensils can occur at different stages of the production and preparation process (Lianou and Sofos, 2007).

While various measures are in place to control infections caused by *L. monocytogenes,* most are focused on limiting its presence in ready-to-eat foods known to pose an infection risk (Kang et al., 2014; Lavieri et al., 2014; Manios and Skandamis, 2014). However, very few studies have examined cross-contamination as one possible source of *L. monocytogenes* infections (Chen et al., 2014; Nakari et al., 2014; Sasaki et al., 2014).

Listeria monocytogenes can be found in decaying vegetables, milk, spices and herbs (Di Pinto et al., 2010; Hassan and Altalhi, 2013; Miya et al., 2010; Schoder et al., 2011) and can caused epidemics in developed nations, such as Belgium and Switzerland (Bula et al., 1995; Weinmaier et al., 2013; Yde et al., 2012). The spread of *L. monocytogenes* via cross-contamination may lead to the contamination of hands and ready-to-eat foods. While cooking and other heat treatments can prevent infection by *L. monocytogenes* (Schoder et al., 2011), this does not apply to cheese, tuna, salads, sushi and other ready-to-eat products. Moreover, as food retail services are not subjected to the same stringent

regulatory framework as the food-processing sector (Lianou and Sofos, 2007), they can be an important source of cross-contamination and spread of *L. monocytogenes*.

L. monocytogenes, a facultative intracellular pathogen, can invade and replicate inside different human cells (Arnett et al., 2014; Lepe et al., 2014; Perry and Higgins, 2013) and can spread from one cell to another without becoming extracellular in the process (Czuczman et al., 2014). Thus, monitoring its presence is of paramount importance in preventing serious food-borne infections.

Presently, no official statistics on the incidence of listeriosis in Saudi Arabia exist. In addition, although the ability of *L. monocytogenes* to contaminate food retailers' surfaces and shoppers' plastic bags is not available. Contamination of the food retailers' surfaces and shoppers' plastic bags can allow *L. monocytogenes* to reach ready-to-eat foods and cause infections.

In order to address this gap in the extant knowledge, in the present study, we determine the prevalence of *L. monocytogenes* in butcheries, supermarkets and small grocery shops by sampling food cutting boards, conveyer belts, handheld shopper baskets and plastic bags at these outlets. Our findings can provide a useful baseline data for regulatory guidelines important for the safety of food outlets. This is of particular importance, as we also detected the spread of *L. monocytogenes* through plastic bags and handheld baskets during the purchasing process. The analyses reported here are based on the samples obtained from butcheries, groceries and supermarkets located in Albaha, Abha and Taif of Saudi Arabia.

2. Material and Methods

Food outlets studied: The data collection was conducted in 48 food outlets, comprising 12 butcheries, 13 supermarkets and 23 small grocery shops from Albaha, Abha and Taif, Saudi Arabia. At these outlets, 96 cutting boards, 216 handheld baskets, 90 checkout conveyer belts and 531 plastic bags carried by the shoppers leaving the food outlets were sampled. Thus, a total of 933 samples were tested.

Food outlet sampling: The aforementioned sampling process was conducted between March and December 2011, resulting in 933 samples. All sampled fomites, except the plastic bags, were tested for *Listeria* spp. by swabbing. The swabs (TRANSWAB®, England) were carried on ice to the laboratory within 2 hours of the sampling process and were inspected for presence of *Listeria* spp. and *L. monocytogenes* immediately.

Sampling plastic bags: Plastic bags carried by the leaving shoppers were sampled from each outlet given a total of 531 samples. The bags were sampled by cutting a 2 cm^2 piece from the bottom of each bag with a sterile scalpel. Each sample was used to inoculate agar plates directly by placing it on the agar surface. In addition, to determine whether contamination occurred during the purchasing process or during the manufacture and transport, 33 unused plastic bags were also sampled.

***L. monocytogenes* isolation:** The collected swabs and plastic bag pieces were used to inoculate Columbia blood agar (5% sheep blood; bioMerieux®, Marcy l'Etoile, France) and Palcam agar (Oxoid Ltd., Basingstoke, United Kingdom). After 24 hours of incubation at 37 °C, seven presumptive colonies from each plate were collected using a sterile toothpick and subcultured immediately. Each isolate was subjected to several confirmatory tests, including Gram-staining, mobility at 25 °C on mobility agar, catalase test and oxidase test.

The expected *L. monocytogenes* colonies were examined by slide agglutination with antiserum (Denka Seiken, Tokyo, Japan), and subsequently subjected to API *Listeria* (BioMerieux®, Marcy l'Etoile, France) for final conformation, following manufacturer's instructions. Multilocus sequence type (MLST) was used to differentiate isolates into strains. The MLST was based on the previously described methods and genes (*prfA, inlB, inlC, dal, clpP, and lisR*) (Chen et al., 2007; Lomonaco et al., 2008; Zhang et al., 2004).

***L. monocytogenes* antibiotic susceptibility testing**. The disc diffusion technique (Agarwal, 1974) and E-tests (AB Biodisk, Solna, Sweden) were used and the Clinical and Laboratory Standards Institute (CLSI) breakpoints were employed (Institute, 2007).

Determination of *L. monocytogenes* survival rate on plastic bags. *L. monocytogenes* detection limit was determined by seeding 100 µl trypticase soy broth cultures containing 1.2×10^6 colony forming units of *L. monocytogenes* (TLCC, University of Otago, New Zealand). The seeding was performed after the surfaces were sterilized with 70% ethanol and left to dry at room temperature. Next, the seeding inoculum described above was applied by spreading it on the surface using a sterile swab. Then, the surfaces under study were sampled and plated 1, 2, 3, 4, 5, 6 and 7 hours post-inoculation on Columbia blood agar (5% sheep blood; bioMerieux®, Marcy l'Etoile, France). This process was repeated four times and the average number of colony forming units per sample was calculated and reported in the results.

3. Results

Prevalence of *L. monocytogenes*. The 933 samples surveyed comprised 96 cutting boards, 90 conveyer belts, 216 handheld baskets and 531 used plastic bags. In addition, 33 unused plastic bags were sampled as a control, all of which were found free of *Listeria* spp. contamination. In the remaining samples, 98/933 (10.5%) and 47/933 (5.9%) were found positive for *Listeria* spp. and *L. monocytogenes*, respectively. The highest rates of *Listeria* spp. (20.9%) and *L. monocytogenes* (16.3%) were found on the cutting boards sampled in butcheries. In contrast, the shoppers' plastic bags samples at small grocery shops were least contaminated. Overall, the contamination rate of 7.8% and 2.5% for *Listeria* spp. and *L. monocytogenes,* respectively, was found for shoppers' plastic bags (Figures 1 and 2). The contamination of handheld shopper baskets from supermarkets and small grocery shops with *Listeria* spp. (12%) and *L. monocytogenes* (6.5%) was more extensive than that found for conveyer belts (8.9% and 4.4%) and plastic bags (8.4% and 4.2%). In supermarkets, cutting boards were found to be the most contaminated, followed by handheld baskets, conveyer belts and plastic bags (Figure 1 and 2).

However, in small grocery shops, the contamination rate was the highest for the conveyer belts, followed by handheld baskets and plastic bags. The butcheries were the most contaminated food outlet in this study, where the greatest number of contaminated plastic bags was also found (Figure 1 and 2). The high rate of contamination of butcheries, compared to the other food outlets, was statistically significant ($p = 0.05$).

Survival rates of *L. monocytogenes* on different food retailers' fomites. The highest *L. monocytogenes* survival rate on surfaces was found on cutting boards, which were also the most contaminated. This was followed by and lowest on conveyer belts. Therefore, cutting boards were the most contaminated and allowed for the higher survival rates for *L. monocytogenes* (Figure 3). The survival rate on the two other sampled fomites (handheld baskets and plastic bags, for which very similar survival rate was recorded, and finally by conveyer belts (Figure 3).

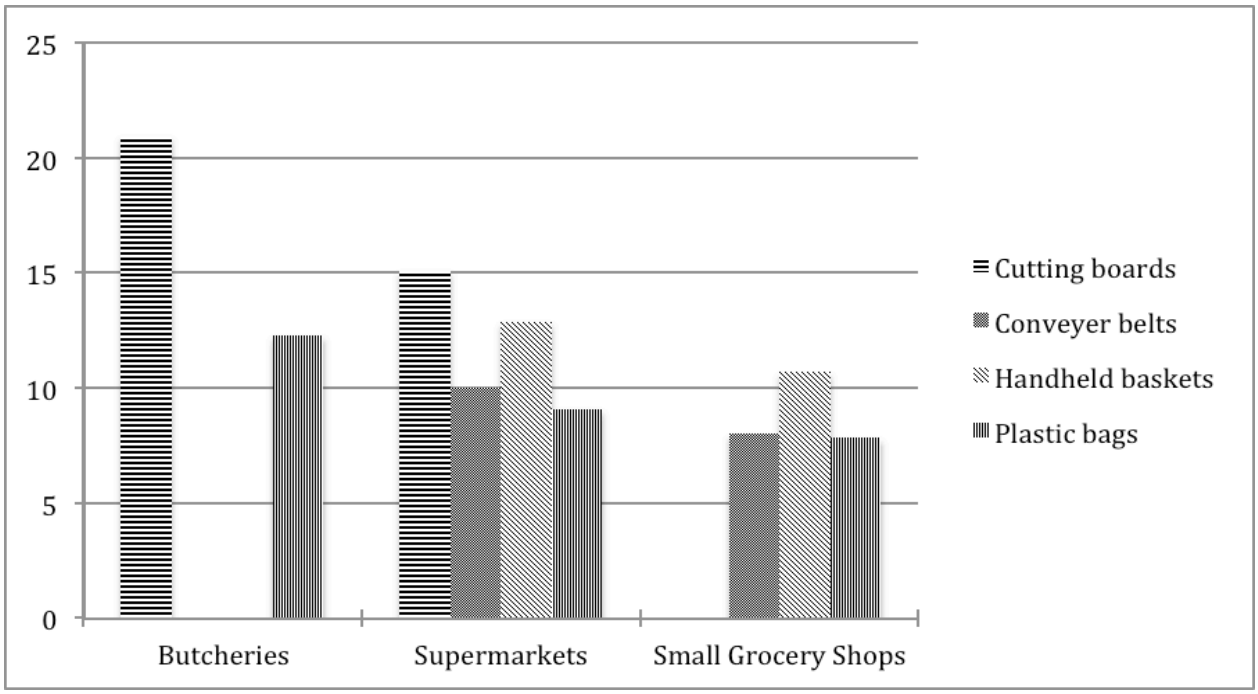

Figure 1: *Listeria* spp. contamination rates in the different food retailers, reported by sample type.

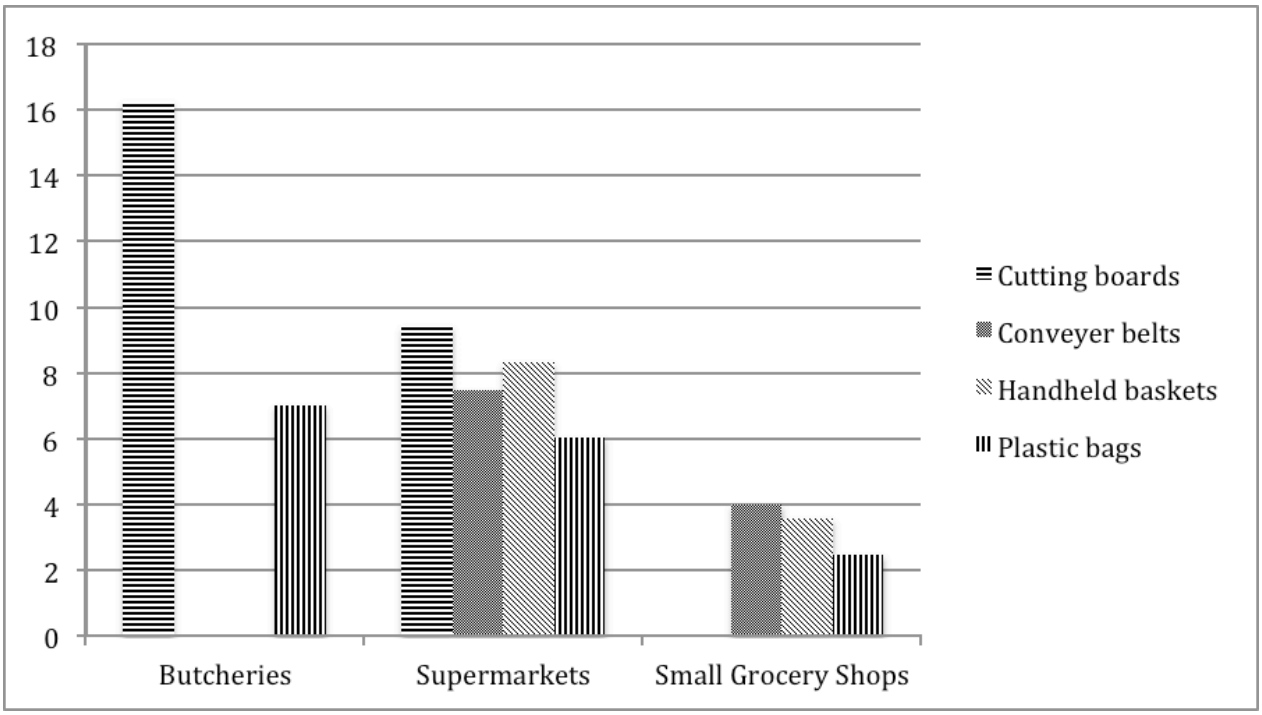

Figure 2: *Listeria monocytogenes* contamination rate in the different food retailers, reported by sample type.

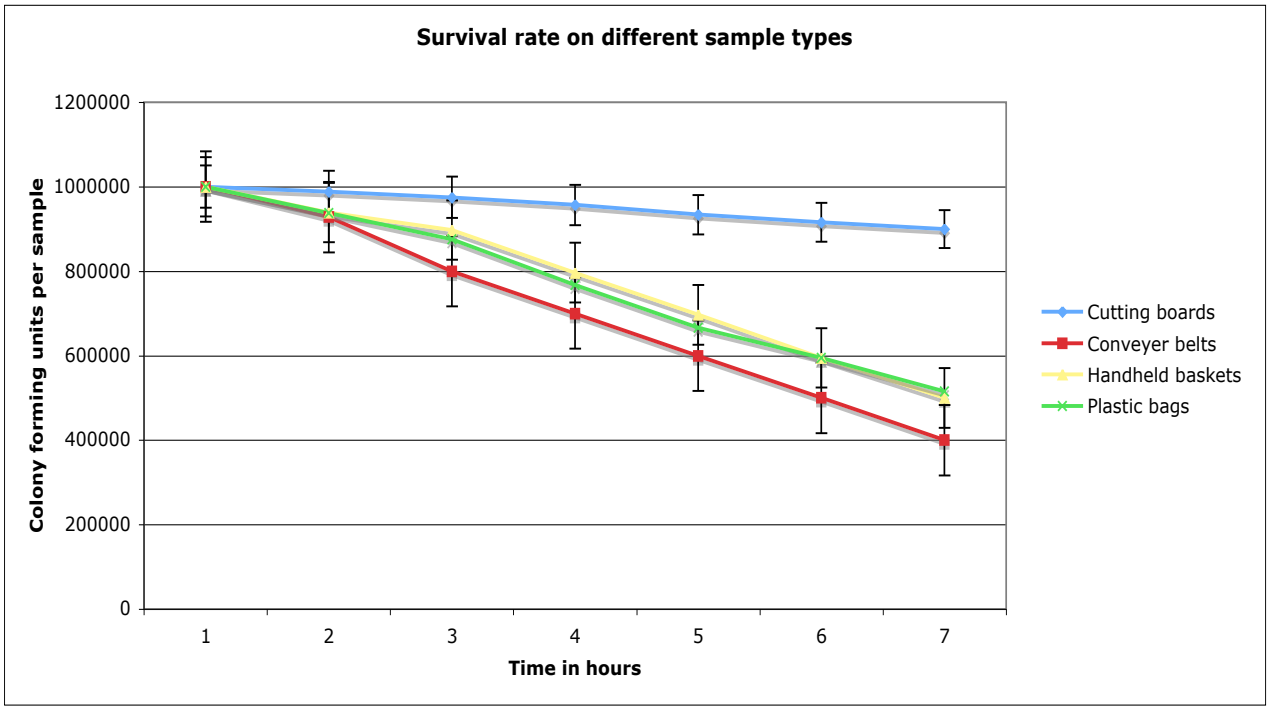

Figure 3: Survival rates of *Listeria monocytogenes* on different fomites.

Antibiotic resistance of *L. monocytogenes*. The isolates confirmed as *L. monocytogenes* that had a different multilocus sequence type (MLST) or a different serotype were considered different strains. The MLST was based on methods and genes (*prfA, inlB, inlC, dal, clpP, and lisR*) previously described in the pertinent literature (Chen et al., 2007; Lomonaco et al., 2008; Zhang et al., 2004). The analyses revealed 108 different strains, which were subjected to antibiotic susceptibility testing, the results of which are presented in Table 1. As can be seen from the data, amoxicillin, gentamicin and chloramphenicol were the most effective antibiotics against the isolated *L. monocytogenes* strains. In contrast, high resistance was common against augmentin, erythromycin and cloxacillin (Table 1).

Table 1: Antibiotic sensitivity profile of different isolates from different food outlets

Isolate	Antibiotic resistant isolates *n* (%)							
	ERY	AMX	CXO	AUG	COT	GEN	TET	CHL
Butcheries cutting boards (*n*=12)	7 (58.3)	0 (0)	9 (75)	9 (75)	9 (75)	1 (8.3)	5 (41.6)	4 (33.3)
Butcheries plastic bags (*n*=12)	6 (50)	0 (0)	6 (50)	9 (75)	5 (41.6)	4 (33.3)	6 (50)	5 (41.6)
Supermarket cutting boards (*n*=12)	6 (50)	0 (0)	7 (58.3)	6 (50)	7 (58.3)	3 (25)	1 (8.3)	3 (25)
Supermarket baskets (*n*=12)	7 (58.3)	0 (0)	6 (50)	11 (91.6)	6 (50)	3 (25)	5 (41.6)	5 (41.6)
Supermarket conveyer belts (*n*=12)	7 (58.3)	1 (8.3)	7 (58.3)	10 (83.3)	7 (58.3)	0 (0)	6 (50)	6 (50)
Supermarket plastic bags (*n*=12)	7 (58.3)	0 (0)	5 (41.6)	9 (75)	6 (50)	2 (16.6)	6 (50)	4 (33.3)
Grocery handheld baskets (*n*=12)	6 (50)	0 (0)	7 (58.3)	11 (91.6)	5 (41.6)	3 (25)	6 (50)	4 (33.3)
Grocery conveyer belts (*n*=12)	7 (58.3)	0 (0)	6 (50)	10 (83.3)	6 (50)	2 (16.6)	4 (33.3)	4 (33.3)
Grocery plastic bags (*n*=12)	6 (50)	0 (0)	7 (58.3)	9 (75)	7 (58.3)	0 (0)	6 (50)	3 (25)

Legend: ERY, erythromycin; AMX, amoxicillin; COX, Cloxacillin; AUG, augmentin; COT, cotrimoxazole; GEN, gentamicin; TET, tetracycline; CHL, chloramphenicol.

4. Discussion

L. monocytogenes is a Gram-positive bacterium. As it is widely distributed in the environment, it is capable of causing infections in humans and many animals, including camels, sheep and cows. While contamination of the animal environment by *Listeria* spp. is common (Borucki et al., 2005; Ho et al., 2007; Nightingale et al., 2004), *L. monocytogenes* has also been isolated from soil surfaces, rotten vegetables and pasture (Fenlon, 1999). *L. monocytogenes* is commonly present in silage where the pathogen can multiply, especially in big bales and animal feces (Husu, 1990). Consequently, dairy farms are a significant reservoir of *L. monocytogenes* (Vilar et al., 2007), even though pasteurization of diary products is an effective method for eliminating *L. monocytogenes*. In particular, food products that cannot be heat-treated can be easily contaminated with cattle manure and *L. monocytogenes* (like green leafy vegetables used in salads). Thus, unpasteurized milk is not the most important route of *L. monocytogenes* contamination, indicating that, in countries where milk pasteurization is compulsory, such as in Saudi Arabia, listeriosis is likely to have a different origin. Moreover, cross-contamination of ready-to-eat foods, such as salads, tuna, sushi, and cheese, is also possible at any stage of manufacturing, retail or consumption.

The examination of cutting boards, baskets, conveyer belts and plastic bags found in various food outlets sampled in this study highlighted the need to acknowledge cross-contamination as a potential source of listeriosis. In this study, the prevalence of *L. monocytogenes* was the highest on cutting boards, followed by baskets and conveyer belts. Moreover, while unused plastic bags were found free of contamination, but after use, through handling by shoppers, they became contaminated at a rate of 2.5% in small grocery shops, 6% in supermarkets and 7% in butcheries.

As our findings confirm, cutting boards, handheld baskets, conveyer belts and plastic bags are potential sources of contamination of ready-to-eat foods, hands and utensils during the purchasing process. Educational approaches for both retail business workers and customers are therefore necessary. The results reported here can assist in designing effective risk prevention methods should be tailored based on the results obtained in this study to target the cutting boards and customer handheld baskets, followed by conveyer belts to reduce plastic bag, hands and ready-to-eat food contamination. This is important to improve the safety of foods being consumed in Saudi Arabia.

The survival rate of *L. monocytogenes* on surfaces was found to be the highest on cutting boards, while it was the lowest on conveyer belts. Therefore, cutting boards present the highest risk for causing listeriosis and require attention from health authorities. This is particularly important for

butcheries and other food outlets where food is in regular contact with cutting boards. Thus the contamination can be limited by their regular disinfection and sanitation. In addition, handheld baskets and conveyer belts need to be regularly cleaned and disinfected to prevent infections and cross-contamination of foods.

Antibiotic resistance profile of the tested isolates indicated high level of resistance to several commonly used antibiotics. While amoxicillin, gentamicin and chloramphenicol were found the most effective antibiotics, high resistance was common against augmentin, erythromycin and cloxacillin. This is valuable information for the medical treatment of infections caused by *L. monocytogenes.*

5. Conclusion

L. monocytogenes is a common contaminate of food outlet surfaces and can be further transmitted via shoppers' plastic bags used to carry purchased items, posing a risk to human health. The average prevalence rate of 10.5% and 5.9% was found for *Listeria* spp. and *L. monocytogenes*, respectively. Cutting boards used in butcheries were the most contaminated, as *L. monocytogenes* was found in 16.3% of the tested samples. In contrast, the lowest rate was found in plastic bags carried by shoppers leaving small grocery shops.

The survival rate of *L. monocytogenes* was also found to be the highest on cutting boards, followed by handheld baskets in supermarkets and small grocery shops. Plastic bags, which are contaminated through contact with contaminated baskets, conveyer belts or cutting boards, were the least contaminated fomite in the three outlets studied.

Finally, amoxicillin, gentamicin and chloramphenicol were found to be the most effective antibiotics against *L. monocytogenes* isolated from food retailers. In contrast, high resistance was common against augmentin, erythromycin and cloxacillin. This data can be used to make informed medical decisions when treating empirically *L. monocytogenes* infections or deciding which antibiotics to use for susceptibility testing.

The findings reported here confirm the need to enhance the sanitization practices to which cutting boards, handheld baskets, and conveyer belts are currently subjected. This effort will prevent the cross-contamination of shoppers' plastic bags, foods and hands of customers and workers in food retailer businesses, thus promoting public health.

Acknowledgements:

The author thanks Prof. John R. Tagg and Prof. Sandy M B Smith for their past guidance; and Dr. Hassan Abdullah Alqumber, Dr. Nedhal Abdullah Alqumber and Dr. Khaled Ahmad Arafa for their support and encouragement.

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References

1. Agarwal, K.C., 1974. Antibiotic sensitivity test by disc diffusion method: standardization and interpretation. Indian J. Pathol. Bacteriol. 17, 149-159.
2. Arnett, E., Vadia, S., Nackerman, C.C., Oghumu, S., Satoskar, A.R., McLeish, K.R., Uriarte, S.M., Seveau, S., 2014. The pore-forming toxin listeriolysin O is degraded by neutrophil metalloproteinase-8 and fails to mediate *Listeria monocytogenes* intracellular survival in neutrophils. J. Immunol. 192, 234-244.
3. Borucki, M.K., Gay, C.C., Reynolds, J., McElwain, K.L., Kim, S.H., Call, D.R., Knowles, D.P., 2005. Genetic diversity of *Listeria monocytogenes* strains from a high-prevalence dairy farm. Appl. Environ. Microbiol. 71, 5893-5899.
4. Bula, C.J., Bille, J., Glauser, M.P., 1995. An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults. Clin. Infect. Dis. 20, 66-72.
5. Chen, M., Wu, Q., Zhang, J., Guo, W., Wu, S., Yang, X., 2014. Prevalence and Contamination Patterns of *Listeria monocytogenes* in Flammulina velutipes Plants. Foodborne Pathog. Dis. doi:10.1089/fpd.2013.1727.
6. Chen, Y., Zhang, W., Knabel, S.J., 2007. Multi-virulence-locus sequence typing identifies single nucleotide polymorphisms which differentiate epidemic clones and outbreak strains of *Listeria monocytogenes*. J. Clin. Microbiol. 45, 835-846.
7. Czuczman, M.A., Fattouh, R., van Rijn, J.M., Canadien, V., Osborne, S., Muise, A.M., Kuchroo, V.K., Higgins, D.E., Brumell, J.H., 2014. *Listeria monocytogenes* exploits efferocytosis to promote cell-to-cell spread. Nature. 509, 230-234.
8. Di Pinto, A., Novello, L., Montemurro, F., Bonerba, E., Tantillo, G., 2010. Occurrence of *Listeria monocytogenes* in ready-to-eat foods

from supermarkets in Southern Italy. New Microbiol. 33, 249-252.
9. Felix, B., Danan, C., Van Walle, I., Lailler, R., Texier, T., Lombard, B., Brisabois, A., Roussel, S., 2014. Building a molecular *Listeria monocytogenes* database to centralize and share PFGE typing data from food, environmental and animal strains throughout Europe. J. Microbiol. Methods. 14, 155-9.
10. Fenlon, D.R., 1999. *Listeria*, listeriosis and food safety, *Listeria monocytogenes* in the natural environment, 2nd Edition. Marcel Decker, New York.
11. Graves, L.M., Hunter, S.B., Ong, A.R., Schoonmaker-Bopp, D., Hise, K., Kornstein, L., DeWitt, W.E., Hayes, P.S., Dunne, E., Mead, P., Swaminathan, B., 2005. Microbiological aspects of the investigation that traced the 1998 outbreak of listeriosis in the United States to contaminated hot dogs and establishment of molecular subtyping-based surveillance for *Listeria monocytogenes* in the PulseNet network. J. Clin. Microbiol. 43, 2350-2355.
12. Hassan, S.A., Altalhi, A.D., 2013. Safety Assessment of Spices and Herbs Consumed In Saudi Arabia: Microbiological Quality and Toxin Production. Life Sci. J. 10, 2819-2827.
13. Ho, A.J., Lappi, V.R., Wiedmann, M., 2007. Longitudinal monitoring of *Listeria monocytogenes* contamination patterns in a farmstead dairy processing facility. J Dairy Sci. 90, 2517-2524.
14. Husu, J.R., 1990. Epidemiological studies on the occurrence of *Listeria monocytogenes* in the feces of dairy cattle. Zentralbl. Veterinarmed. 37, 276-282.
15. Wayne, PA., 2007. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Clinical and Laboratory Standarads Institute. Approved standard M11-A7, 7th ed.
16. Kang, J., Stasiewicz, M.J., Murray, D., Boor, K.J., Wiedmann, M., Bergholz, T.M., 2014. Optimization of combinations of bactericidal and bacteriostatic treatments to control *Listeria monocytogenes* on cold-smoked salmon. Int. J. Food Microbiol. 179, 1-9.
17. Lavieri, N.A., Sebranek, J.G., Brehm-Stecher, B.F., Cordray, J.C., Dickson, J.S., Horsch, A.M., Jung, S., Larson, E.M., Manu, D.K., Mendonca, A.F., 2014. Investigating the Control of *Listeria monocytogenes* on a Ready-to-Eat Ham Product Using Natural Antimicrobial Ingredients and Postlethality Interventions. Foodborne Pathog. Dis. 11, 462-467.
18. Lepe, J.A., Torres, M.J., Smani, Y., Parra-Millan, R., Pachon, J., Vazquez-Barba, I., Aznar, J., 2014. In vitro and intracellular activities of fosfomycin against clinical strains of *Listeria monocytogenes*. Int. J. Antimicrob. Agents. 43, 135-139.
19. Lianou, A., Sofos, J.N., 2007. A review of the incidence and transmission of Listeria monocytogenes in ready-to-eat products in retail and food service environments. J. Food Prot. 70, 2172-2198.
20. Lomonaco, S., Chen, Y., Knabel, S.J., 2008. Analysis of additional virulence genes and virulence gene regions in *Listeria monocytogenes* confirms the epidemiologic relevance of multi-virulence-locus sequence typing. J. Food Prot. 71, 2559-2566.
21. Manios, S.G., Skandamis, P.N., 2014. Control of *Listeria monocytogenes* in the processing environment by understanding biofilm formation and resistance to sanitizers. Methods Mol. Biol. 1157, 251-261.
22. Mead, P.S., Dunne, E.F., Graves, L., Wiedmann, M., Patrick, M., Hunter, S., Salehi, E., Mostashari, F., Craig, A., Mshar, P., Bannerman, T., Sauders, B.D., Hayes, P., Dewitt, W., Sparling, P., Griffin, P., Morse, D., Slutsker, L., Swaminathan, B., 2006. Nationwide outbreak of listeriosis due to contaminated meat. Epidemiol. Infect. 134, 744-751.
23. Miya, S., Takahashi, H., Ishikawa, T., Fujii, T., Kimura, B., 2010. Risk of *Listeria monocytogenes* contamination of raw ready-to-eat seafood products available at retail outlets in Japan. Appl. Environ. Microbiol. 76, 3383-3386.
24. Nakari, U.M., Rantala, L., Pihlajasaari, A., Toikkanen, S., Johansson, T., Hellsten, C., Raulo, S.M., Kuusi, M., Siitonen, A., Rimhanen-Finne, R., 2014. Investigation of increased listeriosis revealed two fishery production plants with persistent *Listeria* contamination in Finland in 2010. Epidemiol. Infect. 24,1-9.
25. Nightingale, K.K., Schukken, Y.H., Nightingale, C.R., Fortes, E.D., Ho, A.J., Her, Z., Grohn, Y.T., McDonough, P.L., Wiedmann, M., 2004. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. Appl. Environ. Microbiol. 70, 4458-4467.
26. Perry, K.J., Higgins, D.E., 2013. A differential fluorescence-based genetic screen identifies *Listeria monocytogenes* determinants required for intracellular replication. J. Bacteriol. 195, 3331-3340.
27. Sasaki, Y., Haruna, M., Murakami, M., Hayashida, M., Takahashi, N., Urushiyama, T., Ito, K., Yamada, Y., 2014. Contamination of poultry products with *Listeria monocytogenes* at

poultry processing plants. J. Vet. Med. Sci. 76, 129-132.
28. Schiller, L.R., 2009. Infectious disease: A germy world-food-borne infections in 2009. Nat. Rev. Gastroenterol. Hepatol. 6, 197-198.
29. Schoder, D., Melzner, D., Schmalwieser, A., Zangana, A., Winter, P., Wagner, M., 2011. Important vectors for *Listeria monocytogenes* transmission at farm dairies manufacturing fresh sheep and goat cheese from raw milk. J. Food Prot. 74, 919-924.
30. Vilar, M.J., Yus, E., Sanjuan, M.L., Dieguez, F.J., Rodriguez-Otero, J.L., 2007. Prevalence of and risk factors for *Listeria* species on dairy farms. J Dairy Sci. 90, 5083-5088.
31. Weinmaier, T., Riesing, M., Rattei, T., Bille, J., Arguedas-Villa, C., Stephan, R., Tasara, T., 2013. Complete Genome Sequence of *Listeria monocytogenes* LL195, a Serotype 4b Strain from the 1983-1987 Listeriosis Epidemic in Switzerland. Genome Announc. 1, 152-12.
32. Yde, M., Naranjo, M., Mattheus, W., Stragier, P., Pochet, B., Beulens, K., De Schrijver, K., Van den Branden, D., Laisnez, V., Flipse, W., Leclercq, A., Lecuit, M., Dierick, K., Bertrand, S., 2012. Usefulness of the European Epidemic Intelligence Information System in the management of an outbreak of listeriosis, Belgium, 2011. Euro. Surveill. 17(38), 20279.
33. Zhang, W., Jayarao, B.M., Knabel, S.J., 2004. Multi-virulence-locus sequence typing of *Listeria monocytogenes*. Appl. Environ. Microbiol. 70, 913-920.

5/25/2014